



# Very Low-Calorie Diet and 6 Months of Weight Stability in Type 2 Diabetes: Pathophysiological Changes in Responders and Nonresponders

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## OBJECTIVE

Type 2 diabetes mellitus (T2DM) is generally regarded as an irreversible chronic condition. Because a very low-calorie diet (VLCD) can bring about acute return to normal glucose control in some people with T2DM, this study tested the potential durability of this normalization. The underlying mechanisms were defined.

## RESEARCH DESIGN AND METHODS

People with a T2DM duration of 0.5–23 years ( $n = 30$ ) followed a VLCD for 8 weeks. All oral agents or insulins were stopped at baseline. Following a stepped return to isocaloric diet, a structured, individualized program of weight maintenance was provided. Glucose control, insulin sensitivity, insulin secretion, and hepatic and pancreas fat content were quantified at baseline, after return to isocaloric diet, and after 6 months to permit the primary comparison of change between post-weight loss and 6 months in responders. Responders were defined as achieving fasting blood glucose  $<7$  mmol/L after return to isocaloric diet.

## RESULTS

Weight fell ( $98.0 \pm 2.6$  to  $83.8 \pm 2.4$  kg) and remained stable over 6 months ( $84.7 \pm 2.5$  kg). Twelve of 30 participants achieved fasting plasma glucose  $<7$  mmol/L after return to isocaloric diet (responders), and 13 of 30 after 6 months. Responders had a shorter duration of diabetes and a higher initial fasting plasma insulin level. HbA<sub>1c</sub> fell from  $7.1 \pm 0.3$  to  $5.8 \pm 0.2\%$  ( $55 \pm 4$  to  $40 \pm 2$  mmol/mol) in responders ( $P < 0.001$ ) and from  $8.4 \pm 0.3$  to  $8.0 \pm 0.5\%$  ( $68 \pm 3$  to  $64 \pm 5$  mmol/mol) in nonresponders, remaining constant at 6 months ( $5.9 \pm 0.2$  and  $7.8 \pm 0.3\%$  [ $41 \pm 2$  and  $62 \pm 3$  mmol/mol], respectively). The responders were characterized by return of first-phase insulin response.

## CONCLUSIONS

A robust and sustainable weight loss program achieved continuing remission of diabetes for at least 6 months in the 40% who responded to a VLCD by achieving fasting plasma glucose of  $<7$  mmol/L. T2DM is a potentially reversible condition.

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Type 2 diabetes (T2DM) has reached epidemic proportions, affecting 9.2% of the U.S. population and costing the country \$322 billion in 2012 (1). The personal cost is enormous in terms of vision loss, amputation, and premature cardiovascular disease. The inevitably progressive nature of the disease has appeared beyond question, as successive large studies have confirmed clinical experience of inexorably worsening glucose control (2,3). At diagnosis, patients are advised to accept having a lifelong disease so that they can cope with T2DM (4). Sequential addition of therapies is required, and within 10 years of diagnosis, 50% of individuals are on insulin therapy (3).

However, restoration of normal glucose control is possible after weight loss in some individuals with T2DM (5–8). Although most commonly seen after bariatric surgery, reversal of diabetes can occur after any sharp decrease in calorie intake (9,10). In short-duration T2DM, fasting plasma glucose becomes normal within days on a very low-calorie diet (VLCD) because of a rapid decrease in liver fat and return of normal hepatic insulin sensitivity, and normal  $\beta$ -cell function returns over 8 weeks (11). If the demonstrated normalization of hepatic insulin sensitivity and  $\beta$ -cell function could be maintained in the longer term, this could change the entire approach to T2DM management.

The Counterbalance study (Counteracting BetA cell failure by Long term Action to Normalize Calorie intake) tests the hypothesis that individuals who achieve nondiabetic fasting blood glucose after VLCD would remain normoglycemic during weight stability. The pathophysiological mechanisms underlying blood glucose control over 6 months were defined.

## RESEARCH DESIGN AND METHODS

### Study Design

This prospective, longitudinal, single-center study comprised three phases: VLCD for 8 weeks; a stepped return to isocaloric intake of normal food over 2 weeks; and a structured, individualized weight maintenance program over 6 months. Assessments were carried out before the VLCD, after return to isocaloric eating, and at the end of the 6-month follow-up. The primary outcome measure was fasting blood glucose at

6 months in the group achieving nondiabetic levels after VLCD and return to normal eating, and the primary comparison was the change between post-weight loss and 6 months in responders. The study was an evaluation of the pathophysiological response to dietary change rather than a comparative clinical study of treatment. In this study, responders were defined as achieving fasting blood glucose  $<7$  mmol/L after return to an isocaloric diet. Immediately after the 8-week VLCD, 87% of short- and 50% of long-duration diabetes groups achieved nondiabetic fasting plasma glucose levels (12). The study was designed to define the durability over 6 months of the clinical and pathophysiological changes after VLCD and return to isocaloric eating and did not include a control group maintained on usual therapy.

### Participants

Thirty individuals with T2DM were recruited by advertisement. The study was rapidly oversubscribed, and of the 48 individuals screened, 18 were excluded due to duration of diabetes or HbA<sub>1c</sub>. To maximize detection of an effect of duration of T2DM, individuals with either short-duration ( $<4$  years) or long-duration ( $>8$  years) disease were studied. Inclusion criteria were age  $\geq 25$ –80 years and BMI 27–45 kg/m<sup>2</sup>. Exclusion criteria were recent weight loss of  $>5$  kg; treatment with thiazolidinediones, GLP-1 agonists, steroids, or atypical antipsychotics; HbA<sub>1c</sub>  $>9.5\%$  (80 mmol/mol); serum creatinine  $>150$   $\mu$ mol/L; or alcohol intake  $>3$  units/day (women) or  $>4$  units/day (men). Participants discontinued all antidiabetic therapy immediately before the baseline study but remained on their usual lipid-lowering treatment. Antihypertensive medications were decreased as necessary throughout the study. The study protocol was approved by the Newcastle and North Tyneside 2 Ethics Committee (REC 12/NE/0208), and all participants gave informed written consent.

### Experimental Protocol

The VLCD consisted of a liquid diet formula (43% carbohydrate, 34% protein, and 19.5% fat; 2.6 MJ/day [624 kcal/day]; OPTIFAST; Nestlé Nutrition, Croydon, U.K.) taken as three shakes per day. In addition, up to 240 g of nonstarchy vegetables was consumed, making total energy intake 624–700 kcal/day. Participants

were encouraged to drink at least 2 L of calorie-free beverages per day and to maintain their habitual level of physical activity. To maximize adherence, one-to-one support was provided weekly by telephone, e-mail, text message, or face-to-face contact (S.S.). During stepped food reintroduction, shakes were gradually replaced by solid food over 7 days; with one meal replacing a shake every 3 days. Isocaloric intake was determined from resting energy expenditure measured by indirect calorimetry using an open circuit calorimeter (Quark RMR; COSMED, Rome, Italy) and a canopy hood. Studies were conducted a minimum of 6 days after full return to solid foods. The standard threshold for remission of diabetes (fasting plasma glucose  $<7$  mmol/L) was used to define the group of responders (13). In contrast to the initial study (11), the criterion was applied after return to isocaloric eating to avoid an acute dietary effect.

During the 6-month weight maintenance phase, participants were supported by a structured individualized program based on goal setting, action planning, and barrier identification, with monthly reviews (14). The primary goal of this phase was to prevent weight regain by individualized dietary advice guided by weight trajectory. Physical activity was encouraged, but food behaviors were the priority. If fasting plasma glucose exceeded 10 mmol/L on two occasions, hypoglycemic agents were recommenced.

As in an earlier study, participants were excluded if they were unable to achieve weight loss targets of 3.8% body weight at week 1 of the VLCD (11). Only one participant did not meet the weight loss target and left the study after week 1; therefore, 29 of 30 participants completed the VLCD and 6-month weight maintenance phase. All 29 completed data collection at each time point.

### Hepatic Glucose Production and Insulin Sensitivity

After an overnight fast, cannulae were inserted into an antecubital vein for infusion and the contralateral wrist vein for arterialized blood sampling. [6'-2H] glucose (98% enriched; Cambridge Isotope Laboratories, Tewksbury, MA) was used to determine hepatic glucose production (11). Basal rates were calculated during

the last 30 min of the 150-min basal period. An isoglycemic-hyperinsulinemic clamp (insulin infusion rate  $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ) was initiated at 0 min, with isoglycemia selected to ensure that the true metabolic condition of each participant could be observed at each study time point. Whole-body insulin sensitivity was determined during the last 30 min of the 120-min hyperinsulinemic glucose clamp expressed per kilogram of fat-free mass (ffm) corrected for glucose space and urinary loss (11). Muscle insulin sensitivity was calculated as the sum of the glucose disposal rate and basal hepatic glucose production minus the urinary glucose loss. Hepatic insulin resistance index was calculated as the product of basal hepatic glucose production and fasting insulin levels (15).

#### Measurement of Hepatic VLDL<sub>1</sub>-Triglyceride Production Rates

VLDL<sub>1</sub>-triglyceride production rate was measured by accumulation of plasma VLDL<sub>1</sub>-triglyceride during competitive blockade of tissue uptake by excess Intralipid (16,17). After an overnight fast, 20% Intralipid (0.1 g/kg body mass) was injected as a bolus followed by continuous infusion of 10% Intralipid at 0.1 g/kg/h. Plasma samples were collected at eight points over 75 min. After centrifugation and ultracentrifugation to separate plasma, remove chylomicrons and Intralipid, and isolate VLDL<sub>1</sub>, the triglyceride concentration of VLDL<sub>1</sub> was measured. VLDL<sub>1</sub>-triglyceride production rates were calculated from the gradient of the linear increase in concentration over time.

#### Assessment of $\beta$ -Cell Function

At least 60 min after the clamp test, when glucose levels had stabilized to fasting levels, two consecutive 30-min square-wave steps of hyperglycemia (2.8 and 5.6 mmol/L above baseline) were achieved by priming glucose doses followed by variable 20% glucose infusion (18). Blood samples for determination of C-peptide concentrations were obtained every 2 min for the first 10 min, then every 5 min for each step. An arginine bolus was administered during the second step of hyperglycemia to assess maximal insulin secretory capacity, followed by sampling every 2 min for 10 min. Insulin secretion rate was calculated by using a computerized program implementing a regularization method of deconvolution and by using a population

model of C-peptide kinetics as previously described (11).

#### Body Composition and Intraorgan Triglyceride Content

Body composition was determined with a Bodystat1500 (Bodystat Ltd., Isle of Man, U.K.). Magnetic resonance data were acquired by using a 3T Philips Achieva scanner (Philips, Best, The Netherlands) with either a six-channel cardiac array (Philips) or four large surface coils (large and medium flex coils; Philips) if required due to body habitus. Data were acquired by three-point Dixon method, with gradient-echo scans acquired during four 17-s breath holds, as previously described (11). The intraorgan triglyceride percentage was evaluated from regions of interest on two image slices of pancreas and five image slices of liver, defined and averaged by one observer (S.S.). The pancreas triglyceride analysis was carried out while blinded to the participants' details and time point.

#### Analytical Procedures

Plasma hormones and metabolites were measured at a Clinical Pathology Accreditation laboratory (Newcastle upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry). VLDL<sub>1</sub>-triglyceride was analyzed at the Institute of Cardiovascular and Medical Sciences, University of Glasgow, using standard methods (Roche Diagnostics, West Sussex, U.K.). Immediate measurement of  $\beta$ -hydroxybutyrate levels to test dietary compliance was carried out using test strips (Abbott Diabetes Care, Oxfordshire, U.K.).

#### Statistical Analysis

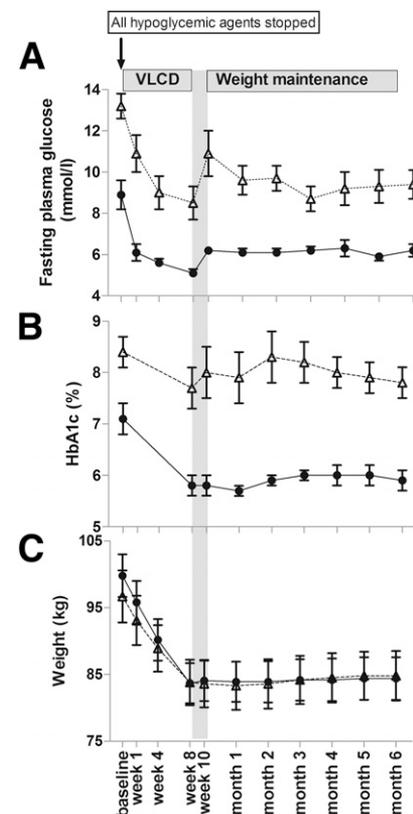
Data are presented as mean  $\pm$  SEM for parametric and median (range) for non-parametric data. Statistical analysis using Student paired and two-sample *t* test, Mann Whitney *U* test, Wilcoxon rank sum test, and Spearman rank correlation as appropriate was performed with the Minitab 16 statistical program (Minitab Inc., State College, PA).

#### RESULTS

In the whole group, weight fell from  $98.0 \pm 2.6 \text{ kg}$  at baseline to  $83.8 \pm 2.4 \text{ kg}$  during the VLCD ( $P < 0.001$ ) and remained at  $84.7 \pm 2.5 \text{ kg}$  after 6 months. After return to isocaloric eating, 40% of participants (12 of 30) achieved a fasting glucose  $<7.0 \text{ mmol/L}$  (responders). After

6 months of weight loss maintenance, 43% (13 of 30) had a fasting plasma glucose  $<7 \text{ mmol/L}$  while off all oral hypoglycemic agents or insulin.

In the responders, fasting plasma glucose fell from  $8.9 \pm 0.7$  to  $6.2 \pm 0.1 \text{ mmol/L}$  ( $P = 0.002$ ) on isocaloric diet post-VLCD and remained constant at  $6.2 \pm 0.3 \text{ mmol/L}$  on no hypoglycemic agents (Fig. 1A). In the nonresponders, fasting plasma glucose fell from  $13.2 \pm 0.6$  to  $10.9 \pm 1.1 \text{ mmol/L}$  ( $P = 0.016$ ) post-VLCD and remained constant at  $9.4 \pm 0.7 \text{ mmol/L}$ . The rise in plasma glucose over the 2 weeks from the end of the VLCD to establishment on an isocaloric diet was significantly greater in nonresponders (Fig. 1A, shaded bar), and six individuals in this group restarted medication during the 6-month weight loss maintenance period (two metformin only, three metformin and sulfonylurea, and one insulin). HbA<sub>1c</sub> remained stable throughout the 6-month period in both groups (responders  $5.8 \pm 0.2$  to  $5.9 \pm 0.2\%$  [ $40 \pm 2$  to



**Figure 1**—Change in fasting plasma glucose (A), HbA<sub>1c</sub> (B), and weight (C) over the study in responders (●) and nonresponders (△). The gray band represents the stepped transition from VLCD to isocaloric eating of solid foods. Data are mean  $\pm$  SEM.

41 ± 2 mmol/mol],  $P = 0.540$ , five individuals <5.7% [39 mmol/mol]; nonresponders 8.0 ± 0.5 to 7.8 ± 0.3% [64 ± 5 to 62 ± 3 mmol/mol],  $P = 0.481$ ) (Fig. 1B). The major improvement in blood pressure and triglyceride and non-HDL cholesterol levels after the VLCD in both responders and nonresponders was maintained over the 6-month weight maintenance period (Table 1). At baseline, 17 participants were taking antihypertensive agents, and these were decreased in dose or stopped in 8 participants, as follows: bumetanide ( $n = 1$ ), nifedipine ( $n = 1$ ), amlodipine ( $n = 1$ ), doxazosin ( $n = 2$ ), ACE inhibitor ( $n = 2$ ), and  $\beta$ -blocker ( $n = 1$ ).

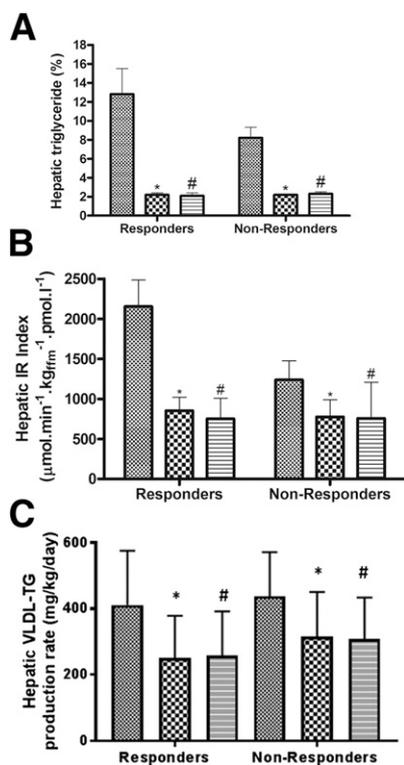
### Clinical Features of Responders Compared With Nonresponders

Achieved weight loss after VLCD was similar between the responders and nonresponders (15.8 ± 0.5% vs. 13.6 ± 0.7%,  $P = 0.06$ ). Weight remained constant over 6 months in both groups (Table 1 and Fig. 1C). The responders ( $n = 12$  [8 males, 4 females]) had a shorter diabetes duration (3.8 ± 1.0 vs. 9.8 ± 1.6 years,  $P = 0.007$ ) and were younger (52.0 ± 2.9 vs. 59.9 ± 2.1 years,  $P = 0.032$ ) than nonresponders ( $n = 17$  [7 males, 10 females]). Responders comprised 9 of 15 of the short-duration group and 3 of 14 of the long-duration group. At baseline, responders had lower fasting glucose (8.9 ± 0.7 vs. 13.2 ± 0.6 mmol/L,  $P < 0.001$ ) and HbA<sub>1c</sub> (7.1 ± 0.3 vs. 8.4 ± 0.3% [55 ± 4 vs. 68 ± 3 mmol/mol],  $P = 0.01$ ). Achieved fasting glucose level after VLCD was positively correlated with diabetes duration ( $R_s$  0.59,  $P = 0.001$ ). Although there was no difference between nonresponders and responders in terms of initial weight or BMI, the total fat mass was higher in nonresponders at baseline ( $P = 0.04$ ) (Table 1). There was no difference in the achieved fasting plasma glucose after the VLCD in individuals with BMI > or <35 kg/m<sup>2</sup> at baseline (8.2 vs. 7.1 mmol/L,  $P = 0.84$ ), with 3 of 10 versus 9 of 19 responders, respectively. Before the study, the responders were on less treatment for diabetes than nonresponders, as follows: diet control (five vs. two); metformin only (six vs. four); metformin and sulfonylurea (one vs. seven); metformin, sulfonylurea, and insulin (zero vs. two); metformin, sulfonylurea, and thiazolidinedione (zero vs. one); and insulin only (zero vs. one).

**Table 1—Fasting anthropometric and metabolic data in responders and nonresponders at baseline, after VLCD and return to isocaloric eating, and after the 6-month weight maintenance period**

	Responders ( $n = 12$ )			Nonresponders ( $n = 17$ )		
	Baseline	After VLCD	After 6 months	Baseline	After VLCD	After 6 months
Weight (kg)	99.8 ± 3.2	84.1 ± 3.1	84.4 ± 3.2	96.7 ± 3.9	83.6 ± 3.5	84.8 ± 3.7
BMI (kg/m <sup>2</sup> )	34.0 ± 0.8	28.6 ± 0.8*	28.7 ± 0.7#	34.4 ± 1.1	29.8 ± 1.1*	30.2 ± 1.1#
Waist-to-hip ratio	0.97 ± 0.02	0.93 ± 0.02*	0.93 ± 0.02#	0.96 ± 0.02	0.91 ± 0.01*	0.92 ± 0.01#
Fat mass (%)	36.2 ± 1.9	30.1 ± 2.0*	31.5 ± 1.9#	42.6 ± 2.2°	37.2 ± 2.0*	40.8 ± 2.5
Serum insulin (mU/L)	20.4 (5.7–48.1)	7.9 (3.4–16.6)*	7.6 (3.1–31.6)#	9.3 (3.9–48.9)°	5.5 (1.4–22.9)*	5.9 (1.2–14.9)#
Serum ALT (units/L)	43 (11–151)	26 (18–42)*	21 (7–27)#	22 (12–61)°	19 (13–47)	18 (9–33)#
Triglycerides (mmol/L)	1.97 ± 0.32	1.25 ± 0.16*	1.15 ± 0.12#	1.30 (0.50–8.10)	1.00 (0.60–2.00)*	1.20 (0.50–3.10)#
Non-HDL cholesterol (mmol/L)	3.6 ± 0.3	2.8 ± 0.3*	2.8 ± 0.3#	3.3 ± 0.3	2.7 ± 0.3*	2.7 ± 0.2#
HDL cholesterol (mmol/L)	1.1 ± 0.1	1.1 ± 0.1	1.4 ± 0.1#	1.3 ± 0.1°	1.3 ± 0.1	1.5 ± 0.1
Basal HGP (mg/kg <sub>frms</sub> /min)	2.6 (2.2–4.0)	2.4 (2.1–3.5)	2.7 (2.4–3.1)	3.3 (2.6–8.1)	3.0 (2.4–4.5)*	3.2 (2.2–8.6)
Hepatic IR index (mmol · min <sup>-1</sup> · kg <sub>frms</sub> <sup>-1</sup> · pmol · L <sup>-1</sup> )	2.15 (0.82–5.95)	0.85 (0.30–1.80)	0.75 (0.31–3.72)	1.24 (0.43–6.60)	0.77 (0.16–2.20)	0.76 (0.17–2.40)
Muscle IS (mg/kg <sub>frms</sub> /min)	5.9 ± 0.4	7.0 ± 0.6	7.2 ± 0.8	8.9 ± 1.3	9.0 ± 0.9	10.4 ± 1.2#
VAT area (cm <sup>2</sup> )	287.0 ± 23.1	191.9 ± 18.9*	179.5 ± 22.3#	289.6 ± 23.7	209.5 ± 22.1*	198.9 ± 4.8#
SAT area (cm <sup>2</sup> )	319.6 ± 31.0	232.0 ± 23.1*	238.6 ± 20.3#	285.4 ± 24.7	223.3 ± 23.5*	219.3 ± 22.8#
Systolic BP (mmHg)	142 ± 5	129 ± 7*	128 ± 5#	159 ± 6	139 ± 5*	143 ± 6#
Diastolic BP (mmHg)	91 ± 2	84 ± 4*	82 ± 2#	90 ± 2	84 ± 2*	85 ± 2#

Data are mean ± SEM or median (range). BP, blood pressure; HGP, hepatic glucose production; IR, insulin resistance; IS, insulin sensitivity; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue. \* $P < 0.05$  for baseline-to-post-VLCD difference. # $P < 0.05$  for baseline-to-month 6 difference. ° $P < 0.05$  for baseline difference between groups.



**Figure 2**—Hepatic triglyceride content (A), hepatic insulin resistance index (B), and hepatic VLDL<sub>1</sub>-triglyceride production (C) in responders and nonresponders at baseline (hatched bars), after VLCD (checked bars), and after 6 months of weight maintenance (striped bars). \* $P < 0.05$  for baseline-to-post-VLCD difference; # $P < 0.05$  for baseline-to-month 6 difference. IR, insulin resistance; TG, triglyceride.

### Change in Plasma Hormones and Metabolites

Responders were characterized by higher baseline serum insulin levels compared with nonresponders (20.4 [5.7–48.1] vs. 9.3 [3.9–48.9] mU/L,  $P = 0.005$ ). This state of relative insulin deficiency in the nonresponders was reflected in higher baseline fasting ketone levels (0.20 [0.10–1.10] vs. 0.10 [0.10–0.30] mmol/L,  $P = 0.02$ ) and free fatty acid levels (0.69 ± 0.04 vs. 0.51 ± 0.05 mmol/L,  $P = 0.01$ ). Plasma insulin levels fell in both groups after the VLCD and remained stable throughout the weight maintenance phase (Table 1). Fasting serum triglyceride levels fell by 32 ± 7% in responders and 18 ± 9% in nonresponders. After 6 months, HDL cholesterol was raised by 27% in responders.

### Liver

Hepatic insulin resistance improved similarly in both groups after the VLCD

(responders 2.15 [0.82–5.95] to 0.85 [0.30–1.80] mmol · min<sup>-1</sup> · kg<sub>ffm</sub><sup>-1</sup> · pmol · L<sup>-1</sup>,  $P = 0.003$ , nonresponders 1.24 [0.43–6.60] to 0.77 [0.16–2.20] mmol · min<sup>-1</sup> · kg<sub>ffm</sub><sup>-1</sup> · pmol · L<sup>-1</sup>,  $P = 0.001$ ) (Fig. 2B). There was no significant change in hepatic insulin resistance index after weight maintenance in either group (0.75 [0.31–3.72] and 0.76 [0.17–2.40] mmol · min<sup>-1</sup> · kg<sub>ffm</sub><sup>-1</sup> · pmol · L<sup>-1</sup>, respectively). At baseline, the responders tended to have greater hepatic insulin resistance (2.15 [0.82–5.95] vs. 1.24 [0.42–6.60] mmol · min<sup>-1</sup> · kg<sub>ffm</sub><sup>-1</sup> · pmol · L<sup>-1</sup>,  $P = 0.060$ ) (Fig. 2B).

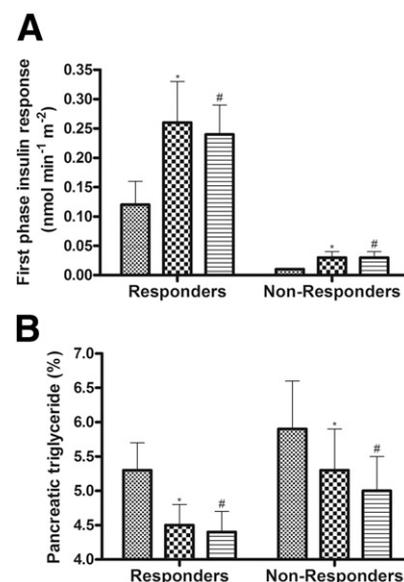
Marked normalization in hepatic triglyceride content was seen in both the responders (12.8 ± 2.7 to 2.2 ± 0.2%,  $P = 0.002$ ) and the nonresponders (8.2 ± 1.1 to 2.2 ± 0.1%,  $P < 0.001$ ) after the VLCD. Serum alanine aminotransferase (ALT) levels decreased only in the responders (43.0 [11.0–151.0] vs. 22.0 [12.0–61.0] units/L,  $P = 0.02$ ), and this was accompanied by a tendency to higher hepatic triglyceride content at baseline compared with nonresponders (12.8 ± 2.7 vs. 8.2 ± 1.1%,  $P = 0.09$ ) (Fig. 2A).

Hepatic VLDL<sub>1</sub>-triglyceride production rate was similar in the two groups at baseline: 411.0 ± 49.5 vs. 437.2 ± 32.4 mg/kg/day in responders vs. nonresponders. It fell by ~20% after VLCD in both groups, remaining stable during weight maintenance (Fig. 2C).

### Pancreas

First-phase insulin response was markedly reduced at baseline in nonresponders compared with responders (0.01 ± 0.00 vs. 0.12 ± 0.04 nmol · min<sup>-1</sup> · m<sup>-2</sup>,  $P = 0.002$ ). Similarly, maximal insulin secretory capacity (baseline to peak insulin secretion rate) was significantly impaired (0.21 ± 0.03 vs. 1.06 ± 0.35 nmol · min<sup>-1</sup> · m<sup>-2</sup>,  $P = 0.008$ ).

First-phase insulin response improved in the responders (0.12 ± 0.04 to 0.26 ± 0.07 nmol · min<sup>-1</sup> · m<sup>-2</sup>,  $P = 0.03$ ), and there was a small increase in the nonresponders (0.01 ± 0.00 to 0.03 ± 0.01 nmol · min<sup>-1</sup> · m<sup>-2</sup>,  $P =$



**Figure 3**—Change in first-phase insulin response (A) and pancreas triglyceride content (B) in responders and nonresponders at baseline (hatched bars), after VLCD (checked bars), and after 6 months of weight maintenance (striped bars). \* $P < 0.05$  for baseline-to-post-VLCD difference; # $P < 0.05$  for baseline-to-month 6 difference.

0.04) (Fig. 3A). There was no change in maximal insulin secretory capacity in either group (responders 1.06 ± 0.35 to 0.81 ± 0.16 nmol · min<sup>-1</sup> · m<sup>-2</sup>,  $P = 0.363$ ; nonresponders 0.21 ± 0.03 to 0.25 ± 0.05 nmol · min<sup>-1</sup> · m<sup>-2</sup>,  $P = 0.30$ ).

First-phase insulin secretion did not change over the weight maintenance period in either responders or nonresponders (0.26 ± 0.07 to 0.24 ± 0.05 vs. 0.03 ± 0.01 to 0.03 ± 0.01 nmol · min<sup>-1</sup> · m<sup>-2</sup>) (Fig. 3). There was no change in maximal insulin secretory capacity.

At baseline, pancreas fat levels were similar in responders and nonresponders (5.3 ± 0.4 vs. 5.9 ± 0.7%,  $P = 0.49$ ). After the VLCD, there was a significant decrease in pancreas fat content in both groups (responders 5.3 ± 0.4 to 4.5 ± 0.3%,  $P = 0.039$ ; nonresponders 5.9 ± 0.7 to 5.3 ± 0.5%,  $P = 0.004$ ) (Fig. 3B). Pancreas fat content remained stable during weight maintenance (4.4 ± 0.3% and 5.0 ± 0.5%).

### Adipose Tissue and Muscle

There was no difference in either visceral adipose tissue or subcutaneous adipose tissue areas between groups at baseline (responders 287.0 ± 23.1

vs.  $289.6 \pm 5.7 \text{ cm}^2$ ,  $P = 0.94$ ; nonresponders  $319.6 \pm 31.0$  vs.  $285.4 \pm 24.7 \text{ cm}^2$ ,  $P = 0.40$ ). Both visceral and subcutaneous adipose tissue areas decreased after the VLCD and then remained constant during the 6-month follow-up (responders: visceral  $287.0 \pm 23.1$  to  $191.9 \pm 18.9$  [ $P < 0.001$ ] then  $179.5 \pm 22.3 \text{ cm}^2$ ; subcutaneous  $319.6 \pm 31.0$  to  $232.0 \pm 23.1$  [ $P < 0.001$ ] then  $238.6 \pm 20.3 \text{ cm}^2$ ; nonresponders: visceral  $289.6 \pm 23.7$  to  $209.5 \pm 22.1$  [ $P < 0.001$ ] then  $198.9 \pm 4.8 \text{ cm}^2$ ; subcutaneous  $285.4 \pm 24.7$  to  $223.3 \pm 23.5$  [ $P < 0.001$ ] then  $219.3 \pm 22.8 \text{ cm}^2$ ). There was no significant improvement in muscle insulin sensitivity after the VLCD (responders  $5.9 \pm 0.4$  at baseline,  $7.0 \pm 0.6$  after VLCD, and  $7.2 \pm 0.8 \text{ mg/kg}_{\text{ffm}}/\text{min}$  at month 6; nonresponders  $8.9 \pm 1.3$ ,  $9.0 \pm 0.9$ , and  $10.4 \pm 1.2 \text{ mg/kg}_{\text{ffm}}/\text{min}$ ).

## CONCLUSIONS

We demonstrate that in 40% of study participants who responded to a VLCD by achieving fasting plasma glucose  $<7 \text{ mmol/L}$ , remission of T2DM lasts for at least 6 months. Return to nondiabetic blood glucose levels was characterized by improvement in acute insulin secretion, and this was sustained while off all hypoglycemic agents. Hepatic insulin sensitivity improved in both responders and nonresponders. The structured, individualized weight maintenance program was successful in preventing weight gain.

Weight loss brought about normalization of liver fat content and insulin sensitivity in both responders and nonresponders. Of note, no redistribution of fat was seen to the liver from the subcutaneous or other deposits over 6 months of weight stability, even though the participants remained obese or overweight (Table 1). This finding supports the concept of a personal fat threshold above which adipose tissue cannot store the available triglyceride (19) and has major implications for the management of nonalcoholic fatty liver disease. Achievement of normal liver fat content was accompanied by a 20% decrease in rate of production of VLDL<sub>1</sub>-triglyceride, the lipoprotein responsible for delivery of triglyceride to all extrahepatic cells and tissues. The observed fall in pancreas fat is a secondary consequence of decreased tissue delivery of triglyceride.

The responders differed primarily in having higher baseline plasma insulin levels and a degree of  $\beta$ -cell response to intravenous glucose. Recovery of acute insulin secretory capacity to nondiabetic levels (20,21) was seen in responders and not in nonresponders. The constancy of the arginine-induced insulin response implies persistence of the insulin secretory mechanism in reversible T2DM despite loss of glucose responsiveness. This is consistent with T2DM being a condition of  $\beta$ -cell dedifferentiation rather than  $\beta$ -cell loss. Nonresponders were characterized by evidence of insulin deficiency at baseline and lack of ability to regenerate insulin secretion capacity. In the human pancreas, the magnetic resonance technique detects the total intra- and extracellular triglyceride in exocrine and endocrine cells, and this is decreased uniquely in T2DM after weight loss (20). The importance of pancreas triglyceride in the pathogenesis of T2DM was initially shown in obese rodents, with local lipolysis bringing about fatty acid-mediated inhibition of  $\beta$ -cell function (22). Exposure to even modest concentrations of fatty acids causes marked triglyceride accumulation in human islets in vitro (23). Chronic exposure of  $\beta$ -cells to triglyceride or fatty acids in vitro decreases  $\beta$ -cell capacity to respond to an acute increase in glucose levels (24,25), and if  $\beta$ -cell fatty acid receptors are knocked out, insulin secretion returns to normal (26). In the human pancreas, intracellular fat droplets are widely distributed within the exocrine pancreatic cells in addition to widely scattered isolated adipocytes (27). Local lipolysis will bring about interstitial and intracellular concentrations of fatty acids sufficient to inhibit  $\beta$ -cell function, and the data suggest that removal of the excess fat allows recovery of function. We hypothesize that in the responders, release from fat-mediated inhibition allows expression of a remaining latent capacity for glucose-responsive insulin secretion.

Current concepts of T2DM have been powerfully shaped by several large studies that have demonstrated a steadily increasing requirement for hypoglycemic agents over years (2,28,29). In particular, the inexorable loss of  $\beta$ -cell function observed during the UK Prospective Diabetes Study reinforced the

view of T2DM as irreversible and progressive (3,30). However, progressive weight gain over time occurred during these long-term observations, and hence, the published data have shown T2DM to be irreversible only during chronic positive calorie balance. The present demonstration of ongoing reversal of T2DM (in 41% of the cohort overall or 60% of individuals with short-duration diabetes) is reflected in population data that indicate that T2DM is solely a response to overnutrition. Ready access to low-cost food is uniformly accompanied by high rates of T2DM (31–33), and when food supply becomes limited for any reason, the prevalence of T2DM falls (34,35).

The question of possible therapeutic application of VLCD for T2DM was raised immediately on publication of the Counterpoint study (36). The present data confirm reversal of T2DM for at least 6 months in those who achieve nondiabetic plasma glucose levels after VLCD. However, the critical question for health care delivery is whether truly long-term reversal of T2DM can be achieved in primary care. To answer this question, a community-based study (DiRECT [Diabetes Remission Clinical Trial]) is now under way in 280 people with T2DM randomized to VLCD with structured individualized weight maintenance or to best-possible guideline-based care. The overall effect of the alternative approaches will be assessed, as the impact of weight loss on blood pressure and lipids is considerable even if plasma glucose levels do not normalize. Being able to stop taking multiple tablets is important to people with T2DM, and the potential associated health care savings are great indeed.

The likelihood of VLCD responders remaining free of diabetes indefinitely must be considered. After media coverage of our earlier study, many people with T2DM reversed their own diabetes (37). For such motivated individuals who avoid weight regain, maintenance of normoglycemia for up to 3 years has been reported (37,38). Follow-up at 4 years of the Look AHEAD (Action for Health in Diabetes) study with only 8.6% weight loss achieved a remission of diabetes in 7.3% of the intervention group (39). Because progression of long-term complications of diabetes relates to ambient blood glucose control,

urable reversal of diabetes would be expected to be associated with long-term health. The effect of a period of normoglycemia confers substantial benefits in decreasing the risk of complications, even if hyperglycemia recurs (40). Whether blood glucose control normalized, a major benefit in vascular risk was achieved in terms of reduction of blood pressure and blood lipids. Long-term prospective study of VLCD followed by a weight maintenance program is now required to define overall benefit.

The intense motivation to return to normal health in a proportion of people with T2DM has not been widely recognized. Such individuals respond readily to simple, unambiguous advice to lose weight (37). For those people who have repeatedly failed to lose weight over many years, this approach is much less likely to succeed. Severely obese subjects are selected for bariatric surgery after all other methods to lose weight have failed, and this group is appropriately treated. However, ~50% of newly diagnosed individuals in the U.K. have a BMI <30 kg/m<sup>2</sup>, and in the UK Prospective Diabetes Study, 36% had a BMI <25 kg/m<sup>2</sup> (19). The overall proportion of people with T2DM who will be able to succeed in the significant long-term lifestyle modification required for VLCD and subsequent weight maintenance with ongoing support remains to be determined.

The VLCD was found to be acceptable as indicated by the low dropout rate in both this and the previous study (11). The principal reason reported was the absence of hunger at this level of calorie intake. The main difficulty was readjusting to normal eating after the VLCD, and this was mitigated by definitive prescription of food type and amount during the food reintroduction and weight maintenance phase. Of note, the need to become used to eating approximately one-third less than previously had been explained in advance. The weight maintenance program, with its clear focus on calorie restriction, individual identification of potential barriers, and monthly contact with S.S. was successful in avoiding weight gain during the 6-month follow-up period. The separate effects of very low-calorie intake itself and change in underlying pathophysiology were defined by the rise in plasma glucose before and after return to isocaloric eating (Fig. 1).

The limitations of the study must be considered. Fewer than one-half of participants (12 of 30) were classified as responders based on achieving a fasting glucose of <7 mmol/L on no antidiabetic medication treatment at the time point immediately after return to isocaloric diet. The study had a small sample size, although the effect size was larger than in pharmacological studies of one or more hypoglycemic agents (41), and the results are definitive. The group size was determined by our previous observations (11) to achieve the specific aims of the study, to examine whether those who achieve nondiabetic fasting blood glucose after VLCD would remain normoglycemic during weight stability, and to determine underlying mechanisms. This was not primarily a treatment trial but rather a pathophysiological study to achieve proof of concept. Six-month follow-up is sufficient to detect any redistribution of fat stores during an isocaloric diet, although longer duration studies are required to define effectiveness as a routine clinical treatment. The group studied was representative of the wider T2DM population, predominantly Caucasian in the northeast of England. Study of other ethnic groups is required. The heterogeneous group studied represents the spectrum of individuals with T2DM who may wish to undertake calorie restriction. A gold standard insulin secretion test was used rather than a test meal to define the acute insulin response because loss of this parameter is a characteristic of T2DM.

T2DM can now be understood to be a metabolic syndrome potentially reversible by substantial weight loss, and this is an important paradigm shift. Not all people with T2DM will be willing to make the changes necessary, but for those who do, metabolic health may be regained and sustained in just under one-half. The observations carry profound implications for the health of individuals and for the economics of future health care.

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